昆虫学报 *Acta Entomologica Sinica*, October 2003, 46 (5): 605 - 608 ISSN 0454-6296

Biotype identification of the populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) in China using RAPD-PCR

QIU Bao-Li[1], REN Shun-Xiang[1]*, WEN Shuo-Yang[1], Nasser S. MANDOUR[1,2]

(1. Entomology Department, South China Agricultural University, Guangzhou 510642, China;
2. College of Agriculture, Suez Canal University, Ismalia, Egypt)

Abstract: Twenty-three *Bemisia tabaci* (Gennadius) populations on vegetables, ornamental plants and weeds were collected from 23 different cities of 14 provinces in China. Biotypes of the different populations were identified and differentiated using a RAPD PCR method with a primer H_{16}. The results showed that 17 whitefly specimens from 11 provinces were B biotype. The data is a good contribution in the distribution and control of *Bemisia tabaci* B-biotype in China.

Key words: *Bemisia tabaci*; biotype; identification; RAPD-PCR; China

The whitefly *Bemisia tabaci* (Gennadius), also known as sweet potato, cotton or tobacco whitefly, has become one of the most serious agricultural pests in many parts of the world in the past few years (Perring *et al*., 1993). The whitefly causes damage through feeding, plant viruses transmission, fouling with honeydew and sooty mould. The losses attributed to this pest exceeds $300 million each year in the world (De Barro and Driver, 1997). The actual number of biotypes of *B. tabaci* is unknown, but at least 24 different biotypes have been identified (Perring, 2001), of these, A and B biotypes are the most serious and widely distributed ones. The B biotype is difficult to control because of the high resistance to most of the insecticides in sales, its wide host range, and rapid rate of development and reproduction (Costa *et al*., 1993; De Barro and Driver, 1997).

In China, *B. tabaci* has been a serious pest since the middle of 1990's, and has been found in more than 20 provinces in China (Luo and Zhang, 2000; Ren *et al*., 2001). In Guangdong, a province of South China, there are about 180 host plants of *B. tabaci*, which lives mainly on vegetables such as tomato, cucumber, eggplant, and ornamentals such as poinsettia and hibiscus (Qiu *et al*., 2001). In this paper, we identified the B biotype from other biotypes of *B. tabaci* by RAPD-PCR method, thus we can draw the distribution of *B. tabaci*, and that will be useful for designing effective strategy to control this pest in future.

1 MATERIALS AND METHODS

1.1 Whitefly source

Whiteflies used in this study were collected from 23 different areas of 14 provinces in China (Table 1). One couple from each place was selected to mate and reproduce on cucumber (*Cucumis sativas* L.) host plants, and then the offspring were collected and deposited in 80% alcohol.

1.2 Sample preparation

All the specimens (nymphs) were washed briefly in sterile double distilled water to remove alcohol before homogenization. Each specimen (about 5 - 8 whiteflies) was put into a 1.5 mL microcentrifuge tube, and 200 μL lysis buffer (1% SDS, 10 mmol/L Tris-HCl, 25 mmol/L NaCl pH 8.0, 25 mmol/L EDTA, 200 mg/mL proteinase K) was added to the tube. The homogenate was incubated at 55℃ for 3 h. After incubation, same volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the microcentrifuge tube to extract

---

基金项目：国家“十五”计划项目（2001BA509B0604）；广东省自然科学基金项目（010312）；广东省自然科学基金重点项目（9622035-02）

作者简介：邱宝利，男，1973年生，博士，主要从事生物防治和昆虫分子进化研究，E-mail: baileyqiu@yahoo.com.cn

* 通讯作者 Author for correspondence, E-mail: rensxcn@yahoo.com.cn

收稿日期 Received: 2002-11-14; 接受日期 Accepted: 2003-01-27

whitefly DNA. Then tubes were shaken gently by hands for 5 min, after which the homogenate was centrifuged for 5 min, 5 000 r/min, and the superstratum was moved to a new microcentrifuge tube. DNA extraction was replicated for 3 times for purification, after which DNA samples were stored in TE (pH 7.8) at -20℃.

Table 1 The biotypes of whiteflies from different areas in China

Population code	Collection location	Host plant	Collection date	Biotype
A	Haidian, Beijing	Tomato	Aug. 2000	B
B	Taian, Shandong	Tomato	May 2000	B
C	Qingdao, Shandong	Tomato	May 2000	B
D	Danzhou, Hainan	Tomato	Feb. 2001	B
E	Fuzhou, Fujian	Sweet potato	Mar. 2000	B
F	Nanning, Guangxi	Tomato	Sep. 2000	B
G	Yichang, Hubei	Hibiscus	Oct. 2000	non-B
H	Kunming, Yunnan	Tomato	Nov. 2000	non-B
I	Yangzhou, Jiangsu	Tomato	June 2001	B
J	Hefei, Anhui	Tomato	Aug. 2001	B
K	Wanzhou, Chongqing	Variegated leaf croton	Sep. 2001	B
L	Xiangtan, Hunan	Eggplant	July 2001	non-B
X	Guiyang, Guizhou	Cucumber	Sep. 2001	B
N	Nanchang, Jiangxi	Eggplant	Sep. 2000	B
O	Guangzhou, Guangdong	Tomato	Dec. 1999	B
P	Zhongshan, Guangdong	Poinsettia	Feb. 2000	B
Q	Foshan, Guangdong	Poinsettia	Apr. 2000	B
R	Shenzhen, Guangdong	Eggplant	May 2000	B
S	Meizhou, Guangdong	Tomato	May 2000	non-B
T	Yangshan, Guangdong	Hibiscus	June 2000	B
U	Yingde, Guangdong	Poinsettia	Aug. 2000	non-B
V	Xinfeng, Guangdong	Poinsettia	Nov. 2000	B
W	Shixing, Guangdong	Weeds	Oct. 2000	non-B

1.3 RAPD-PCR reaction

According to De Barro and Driver (1997), the best primer for *Bemisia tabaci* B biotype identification, H_{16}(5′TCTCAGCTGG 3′), was used, which was bought from OPERON Company. The reaction volume of 25 μL were used with final concentration of 14 μL double distilled water, 10 × PCR buffer 2.5 μL, dNTPs 0.25 mmol/L, Mg^{2+} 0.25 mmol/L, H_{16} primer 20 ng, Taq DNA polymerase 1.5 unit and 10 - 20 ng DNA. Amplification was done in a Eppendorf thermocycler using the following parameters: one cycle of 5 min at 94℃, 2 min at 40℃ and 3 min at 72℃, followed by 39 cycles of 1 min at 94℃, 1.5 min at 40℃ and 2 min at 72℃. Amplified materials were electrophoresed respectively using 1.5% agarose at 8 V/cm for 2 h with 1 × TAE, and the gel was dyed with ethidium bromide. A 100 bp molecular weight marker was used in each gel. Bands on the gels were made visible using a UV light and photographed.

2 RESULTS

2.1 The character of *Bemisia tabaci* B biotype

Whitefly undergoes arrhenotokous parthenogenesis, males are haploid while females are diploid. Therefore, male have half the nuclear genetic material of female. In this study, the H_{16} primer was used in RAPD PCR reaction. The characteristic electrophoretic pattern of *B. tabaci* B biotype (collected from tomato plants, Guangzhou) is showed in Fig.1. The results showed that the amplification was successful, and there are three special bands between 300 bp and 600 bp, which are unique to the B biotype. The characteristic electrophoretic pattern agrees with the data of De Barro and Driver (1997).

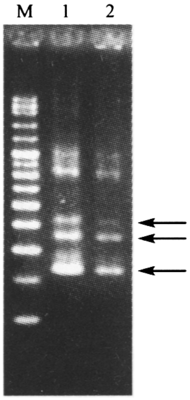

Fig. 1 RAPD-PCR profiles of *B. tabaci* using primer H_{16}

M: 100 bp molecular weight marker; labeled 1 and 2 are both with electrophoretic pattern of B biotype

2.2 The biotypes and distributions of whiteflies

In this study, RAPD PCR replications of the same whitefly specimen were good, and the results of different whiteflies were various. The biotypes of *B. tabaci* collected from 23 areas of 14 provinces are shown in Table 1. The results have shown that 17 whitefly specimens from 17 areas of 11 provinces are B biotype. The RAPD amplification with primer H_{16} of different whiteflies specimens are shown in Fig. 2.

3 DISCUSSION

Before the 1990s in China, the prevalent and most serious species of whiteflies was *Trialeurodes vaporariorum* (Westwood); however, nowadays, *Bemisia tabaci* has replaced the former since 1990s. It has widely distributed in more than 25 provinces in China, including Beijing, Xinjiang, Shandong, Tianjin, Hebei, Guangdong, Fujian, Hainan, etc. (Luo and Zhang, 2000; Ren *et al*., 2001). The main host plants are greenhouse vegetables, ornamental plants and other economical crops. The annual damage caused by *B. tabaci* is high, particularly by the B biotype (Luo and Zhang, 2000).

Biotypes of *B. tabaci* in Beijing, Xinjiang, Guangzhou and some other provinces have been identified by Luo *et al*. (2002) and Wu *et al*. (2002) using mtDNA CO Ⅰ sequencing or RAPD-PCR. In our study, 17 of 23 whitefly populations from different places are B biotype. Our results have shown that the distribution of *B. tabaci* biotypes depends on its host plants, for example, most of the populations on vegetables (*e.g.*, cucumber, eggplant, collard, tomato and some melons) and ornamental plants (*e.g.*, poinsettia and hibiscus) are B biotype. Moreover, the results have also shown that B biotype is present in the big cities or seaside cities with developed transportation systems; therefore, whitefly is migrating during the transportation

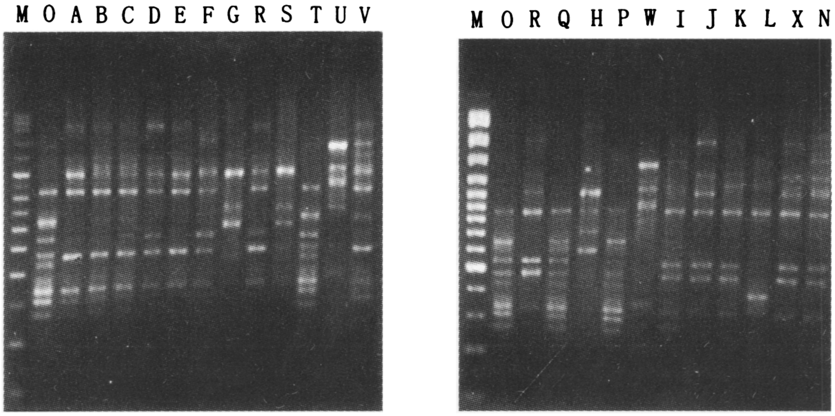

Fig. 2 RAPD-PCR amplification with primer H_{16} to whitefly specimens

M: 100 bp molecular weight marker

of ornamentals, flowers or other seedlings. In the big cities of Guangdong Province, such as Guangzhou, Shenzhen, Foshan, the populations of *B. tabaci* are B biotype; while in the mountainous area, such as Meizhou, Shixing, Yingde, *B. tabaci* are non-B biotype. The origin of *B. tabaci* B biotype is still unknown; study on the biotypes of *B. tabaci* may help us to find the way to solve this problem.

Among the different biotypes of *B. tabaci*, the B biotype is the most serious one, due to its high resistance ability to the chemical pesticides, its broad host range, also for its high reproductive potential and migration ability. It could be concluded that *B. tabaci* B biotype is in ascending in China and it can cause serious damage in agriculture; therefore, much attention should be paid for monitoring this pest and due to its ability to build up a rapid resistance to pesticides in sales, and other effective methods to control this pest, such as biological control, physical control as well as cultural control, should be used.

References

Brown J K, Fohlich D R, Rosell R C, 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a new species complex. *Ann. Rev. Entomol.*, 40: 511 - 534.

Costa H S, Brown J K, Sivasupramanism S, Bird J, 1993. Regional distribution, insecticide resistance, and reciprocal cross between the 'A' and 'B' biotypes of *Bemisia tabaci*. *Insect Sci. Appl.*, 14: 255 - 266.

De Barro P, Driver J F, 1997. Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Austra. J. Entomol.*, 36: 149 - 152.

Luo C, Zhang Z L, 2000. Study progress on *Bemisia tabaci* (Gennadius). *Beijing Agri. Sci.*, 18 (Suppl.): 4 - 13. [罗晨，张芝利，2000. 烟粉虱 *Bemisia tabaci* (Gennadius) 研究概述. 北京农业科学，18 (增刊)：4 - 13]

Luo C, Yao Y, Wang R J, Yan F M, Hu D X, Zhang Z L, 2002. The use of mitochondrial cytochrome oxidase I (mt COI) gene sequences for the identification of biotypes of *Bemisia tabaci* (Gennadius) in China. *Acta Entomologica Sinica*, 45 (6): 759 - 763. [罗晨，姚员，王戎疆，阎凤鸣，胡敦孝，张芝利，2002. 利用 mtDNA CO Ⅰ 基因序列鉴定我国烟粉虱的生物型. 昆虫学报，45 (6)：759 - 763]

Perring T M, Cooper A D, Rodriguez R J, Farrar C A, Bellows T S, 1993. Identification of a whitefly species by genomic and behavioral studies. *Science*, 259: 74 - 77.

Perring T M, 2001. The *Bemisia tabaci* species complex. *Crop Protection*, 20: 725 - 737.

Qiu B L, Ren S X, Sun T X, Lin L, Kuang Z B, 2001. Investigation of host plants of *Bemisia tabaci* (Homoptera: Aleyrodidae) in Guangzhou area. *J. South China Agric. Univer.*, 22 (4): 43 - 47. [邱宝利，任顺祥，孙同兴，林莉，邝灼彬，2001. 广州地区烟粉虱寄主植物调查初报. 华南农业大学学报，22 (4)：43 - 47]

Ren S X, Wang Z Z, Qiu B L, Xiao Y, 2001. The pest status of *Bemisia tabaci* in China and non-chemical control strategies. *Entomologica Sinica*, 8 (3): 279 - 288.

Wu X X, Hu D X, Li Z X, Shen Z R, 2002. Using RAPD-PCR to distinguish biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) in China. *Entomologica Sinica*, 9 (3): 1 - 8.

利用 RAPD-PCR 方法鉴定我国烟粉虱的生物型

邱宝利[1]，任顺祥[1]，温硕洋[1]，Nasser S. MANDOUR[1,2]

(1. 华南农业大学资源环境学院昆虫学系，广州 510642;
2. College of Agriculture, Suez Canal University, Ismalia, Egypt)

摘要：收集了国内北京、山东、广东和海南等14个省市23个地区的蔬菜、园林花卉和杂草上的23个烟粉虱 *Bemisia tabaci* (Gennadius) 种群，根据报道合成了一个随机引物 H_{16}，运用 RAPD-PCR 技术对所收集的烟粉虱种群进行了生物型鉴定。结果表明在23个烟粉虱种群中，有17个种群同属于危害严重的“B生物型”，这些种群主要分布在交通便利的城市或沿海地区，而非B生物型则主要分布在交通不便的山区或内陆。对烟粉虱B生物型的分布与寄主植物和环境条件进行了初步的探讨。

关键词：烟粉虱；生物型；鉴定；RAPD-PCR；中国

中图分类号：Q965 **文献标识码**：A **文章编号**：0454-6296 (2003) 05-0605-04